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# Vascular repair and regulation in kidney disease: Overview

The International Society of Nephrology (ISN) Forefronts in Nephrology Conference entitled "Vascular Repair and Regulation in Kidney Disease" was held September 30, 2004, to October 2, 2004, in Dresden, Germany.

In the opening presentation, Michael Goligorsky (Valhalla, New York) considered the concept of endothelial dysfunction in historical perspective (see following article). He also presented in-depth recent findings concerning the way(s) by which homocysteine, asymmetric dimethylarginine (ADMA), advanced glycation end products (AGEs), and 3-nitrotyrosine–modified proteins are instrumental in contributing to endothelial dysfunction.

Kerstin Amann (Erlangen-Nürnberg, Germany) analyzed the ramifications of uremia-related atherosclerosis in experimental animals. In rats, endothelin antagonists were of particular usefulness in preventing artherosclerotic uremic arterial remodeling. In addition in her experiments, bone morphogenetic protein-7 (BMP-7) was able to prevent vascular calcification. Uremia was also associated with increased plaque size, increased oxidative stress in the vessel wall, and an augmented expression of adhesion molecules on the endothelium.

Bengt Lindholm (Stockholm, Sweden) discussed the incidence of arteriosclerosis in end-stage renal disease (ESRD), the multiple factors involved and the putative roles of specific molecules such as C-reactive protein (CRP), interleukin (IL)-6, and myeloperoxidase (MPO) in causing inflammation and arteriosclerosis. As for treatment, Dr. Lindholm mentioned statins, angiotensin-converting enzyme (ACE) inhibitors, peroxisome proliferators-activated receptor-$\gamma$ (PPAR-$\gamma$) activators, tocopherols, and N-acetylcysteine.

The following session was dedicated to oxidative stress. Michael Wolin (Valhalla, New York) described external conditions, such as age and hypertension as causative factors increasing superoxide formation (see following article). He analyzed the different nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and their regulation.

**Key words:** nitric oxide, oxidative stress, endothelial progenitor cells.



Ralf Brandes (Frankfurt, Germany) said that all customary methods of determination of oxidative stress (direct measurements of radicals, determination of reaction products of radicals, effects on signal transduction, effects on gene expression, assays for antioxidative capacity, activity of antioxidative enzymes, activity of radical generating enzymes) are unsatisfactory if used alone (see following article). He suggested that at least two independent methods were needed to make valid statements about oxidative stress.

Thomas Münzel (Mainz, Germany) demonstrated endothelial dysfunction in an animal model of hypertension due to angiotensin infusion. He showed that the latter increased protein kinase C (PKC) activity leading to enhanced NADPH oxidase activity and more superoxide generation. This in turn leads to endothelial nitric oxide synthase (eNOS) uncoupling via generation of peroxinitrite and ADMA. eNOS uncoupling was the cause of the endothelial dysfunction. Incidentally he also addressed nitrate tolerance. He advanced data showing that nitrate tolerance too was attributable to eNOS uncoupling, which was due to $THB_4$ "deficiency" from tetrahydrobiopterin ($THB_4$) oxidation, both amenable to treatment with folic acid and $THB_4$.

Jan Galle (Würzburg, Germany) discussed oxidative stress in chronic renal failure. Amongst the causes of oxidative stress he mentioned endogenous stimuli [angiotensin II, oxidized low-density lipoprotein (oxLDL), lipopolysaccharide (LPS), AGEs, hyperhomocysteinemia] and exogenous ones (endotoxin from dialysate and blood/membrane contact). He indicated specifically that oxLDL was damaging in that it caused endothelial dysfunction via NADPH oxidase activation, calcium sensitization of vascular smooth muscle cells (VSMCs) via rho kinase activation and apoptosis of vascular cells by direct superoxide action. Beyond statins and reactive oxygen species (ROS) inhibitors, he recommended the use of ultrapure water, biocompatible membranes, elimination of smoking, optimization of hygienic conditions, correction of anemia, and avoidance of iron overload.

Another session focused on nitric oxide. Patrick Vallance (London, United Kingdom) discussed the biochemistry of ADMA and its relevance in uremia and cardiovascular disorders. He suggested that ADMA is effective in changing genetic programs. For example, ADMA was

shown to alter BMP signaling and osteocalcin at the genetic level.

Jens Passauer (Dresden, Germany) tackled the controversies surrounding the role of nitric oxide in uremic endothelial dysfunction (see following article). He concluded that baseline nitric oxide generation was normal in uremia, whereas agonist-induced nitric oxide generation (e.g., by infusing acetylcholine) was subnormal, if functional testing of forearm resistance vessels was employed. He suggested that eNOS uncoupling or nitric oxide consumption byproducts of oxidative stress might be involved in causing the deficient nitric oxide function.

Ingrid Fleming (Frankfurt, Germany) had been asked to analyze the regulation of eNOS. She concentrated on three aspects: (*1*) shear stress [or vascular endothelial growth factor (VEGF)-induced eNOS activation (involving PT3K and Akt)]; (*2*) bradykinin-induced eNOS activation (calmodulin); and (*3*) the multiple pathways of eNOS down-regulation by oxLDL (involving intracellular redistribution of eNOS, defective calmodulin binding, and dephosphorylation of eNOS on $Thr^{495}$).

Malte Kelm (Düsseldorf, Germany) reviewed evidence on whether there is a circulating pool of nitric oxide. He considers nitrite in human blood—a degradation product of nitric oxide—to be a diagnostic tool, being related to nitric oxide production and in proportion to the number of cardiovascular risk factors in patients. He showed experiments in which an intravenous application of nitric oxide into the brachial artery on one side (in human volunteers) was followed several minutes later first by an increase of measurable circulating nitroso compounds in the contralateral arm followed a short time later by forearm vasodilation on that (contralateral) side. As nitroso compounds possibly storing and transporting nitric oxide, he considered the following: albumin, hemoglobin, and nitrated lipids.

Diabetic nephropathy was also an issue in a separate session. Angelika Bierhaus (Heidelberg, Germany) discussed recent findings, including those from her own laboratory on the AGE/receptor for AGE (RAGE)/nuclear factor-κB (NF-κB) axis. In experimental diabetes mellitus there are now knockout models in the mouse (including a $RAGE^{-/-}$ mouse) that appear to be helpful in further analyzing the role(s) of RAGE and AGE. In these models, Bierhaus' group finds evidence that AGE/RAGE contribute to the development of late diabetic complications, such as neuropathy, which is ameliorated in the $RAGE^{-/-}$ mouse. The renal changes in $RAGE^{-/-}$ diabetic mice have not been demonstrated, yet. However, blockade of RAGE by "treatment" with soluble RAGE given parenterally reduced albuminuria and increased creatinine clearance in a diabetic mouse model. In a similar vein, using blockade of RAGE by s-RAGE or using $RAGE^{-/-}$ knockout models it was shown that denudation induced arteriosclerosis was inhibitable by those maneuvers.

Hi Bahl Lee (Seoul, Korea) described diabetic nephropathy, as it relates to ROS, signaling, and matrix remodeling. He stressed the dramatic increase projected globally until 2030. Present treatments delay onset and progression of diabetic nephropathy but do not stop them from occurring. High glucose induces renal injury by induction of ROS, which, in turn, leads to extracellular matrix (ECM) accumulation. Diabetic glomeruli show intense ROS-related signals. ROS generation in mesangial cells is dependent on (inhibitable?) PKC activation and NADPH oxidase stimulation, both of which appear to be interrelated. ROS are also involved in NF-κB activation in mesangial cells exposed to high glucose. Antioxidants (melatonin) and superoxide dismutase (SOD) protectively inhibited some of the pathologic alterations.

Endothelial cells are involved in renal angiogenesis and glomerular function. Börje Haraldsson (Gothenburg, Sweden) reviewed the role of glomerular endothelium in the glomerular filtration barrier. The glomerular endothelial cell surface coat "glycocalyx" is an important component of this barrier. Production of glycosaminoglycans by endothelial cells is highly dynamic. Impairment of size and charge selectivity of the glomerular filtration barrier due to impaired production of glycosaminoglycans is one feature of endothelial dysfunction. Dr. Haraldsson and his colleagues developed a new heterogenous charged fiber model to estimate size and charge selectivities in vivo. Experimental destruction of glycocalyx by hyaluronidases administered to a mouse model resulted in a remarkable loss of barrier function with proteinuria.

Barbara Ballermann (Edmonton, Canada) described the regulation of glomerular endothelial cell growth (see following article).

Wolfgang Fierlbeck (Frankfurt, Germany) discussed the role of apoptosis during organ development. He focused on the lumen formation of glomerular capillaries. Transforming growth factor-β (TGF-β) plays a crucial role for lumen formation by initiating apoptosis of redundant endothelial cells within the compact endothelial cell mass. A subset of surviving endothelial cells organizes into tubes. Labeling of apoptotic cells within the glomerular capillary with antibodies against endothelial surface markers demonstrated their endothelial origin. Inhibition of TGF-β1 by neutralizing antibodies during organogenesis in vivo resulted in reduced lumen formation of the glomerular capillary and in retention of undifferentiated endothelial cells. Furthermore, the frequency of apoptotic endothelial cells within the capillary lumen was reduced.

Marianne C. Verhaar (Utrecht, The Netherlands) discussed the role of bone marrow–derived cells in

endothelial repair. Using the anti-Thy 1.1 nephritis mouse model they could demonstrate that a significant number of bone marrow–derived cells contribute to the glomerular endothelial cell turnover.

Danilo Fliser (Hannover, Germany) showed that erythropoietin (EPO) is not only the main stimulator of erythropoesis but also contributes to the development of endothelial progenitor cells (EPC). A possible underlying mechanism is the stimulation of protein kinase B (Akt) via the EPO receptor. Akt in turn is known to stimulate eNOS. Recombinant human EPO in a dose that did not augment hematocrit increased the number of circulating EPC in humans as well as their ability to form tubes within matrigel in vitro. Treatment with recombinant EPO in vivo offers the possibility of tissue protection as demonstrated by a prolonged preservation of renal function in the 5/6 nephrectomized rat or the improved neovascularization in the rat after hind limb ischemia.

Ulrich Laufs (Homburg, Germany) reported an increased number of circulating EPC after physical exercise in mice. The training effect was completely abolished by systemic blockade of NOS with L-NMMA and in eNOS knockout mice demonstrating a crucial role of eNOS in EPC regulation. Furthermore, physical exercise improved vascular reactivity in response to the endothelium-dependent vasodilator acetylcholine in these mice. The vasodilatation was nitric oxide–mediated. The authors found an improved neoangiogenesis and a reduced neointima formation after exercise. The stimulating effects of physical training on the number of circulating EPC were confirmed in a study of healthy volunteers as well as in patients with coronary artery disease. However it still remains to be clarified whether circulating EPC are the prerequisite for endothelial repair and intact endothelial function or not.

Carmen Urbich (Frankfurt, Germany) reviewed the function of EPC in patients with arteriosclerosis. She reported stimulatory effects of statin therapy on these cells (see following article).

Marlies Elger (Hannover, Germany) reported the possibility of nephrogenesis and glomerulogenesis in the adult little skate. Obviously, there are persisting renal stem cells in a privileged sector of the adult kidneys of these lower vertebrates.

With a focus on kidney and pancreas Marc Hammerman (St. Louis, Missouri) described metanephroi and offered new possibilities for xenotransplantation of these organs at an early stage of organogenesis (see following article).

Using atomic force microscopy Hans Oberleithner (Muenster, Germany) could demonstrate an increase of endothelial cell surface area and stiffness induced by treatment with aldosterone (see following article). These effects were completely reversed by spironolactone.

Ralf Dechend (Berlin, Germany) reviewed the pathogenesis of preeclampsia. They identified functionally active autoantibodies against the angiotensin II type 1 receptor. They demonstrated an autoantibody induced induction of NADPH oxidase and in turn of NF-κB in the placenta of preeclamptic women. Furthermore, they demonstrated an impaired endothelial function in women with a susceptibility to preeclampsia. Possible causes considered were circulating ADMA, up-regulation of antiangiogenic factors such as sFlt1 (soluble receptor for VEGF) and decreased circulating levels of VEGF. The clinical relevance of these findings has yet to be elucidated.

Martin Tepel (Berlin, Germany) focused on the effects of an antioxidant treatment with acetylcysteine and vitamin E in uremic patients. Uremia is associated with increased production of ROS. Two clinical studies showed a clear benefit of either treatment (acetylcysteine or vitamin E) compared to placebo regarding the composite end point of death, myocardial infarction or intervention for coronary or peripheral artery disease in patients with ESRD. Possible underlying mechanisms are a reduction of oxLDL or of homocysteine.

PETER GROSS, HI BAHL LEE, and FRANK PISTROSCH

*Division of Nephrology, Department of Medicine, University Medical Center, Carl Gustav Carus, Dresden, Germany; and Hyonam Kidney Laboratory, Soon Chun Hyang University, Seoul, Korea*

*Reprint requests to Peter Gross, M.D., Nephrologie, Medizinische Klinik III, Universitätsklinikum C.G. Carus, Fetscherstrasse 74, D-01307 Dresden, Germany.*
*E-mail: peter.gross@mailbox.tu-dresden.de*